(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 July 2014 (17.07.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/107815 A3

(51) International Patent Classification:
C12N 1/21 (2006.01) *C12N 15/87* (2006.01)
C12N 15/74 (2006.01)

(21) International Application Number:
PCT/CA2014/050181

(22) International Filing Date:
6 March 2014 (06.03.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/750,355 8 January 2013 (08.01.2013) US

(72) Inventor; and
(71) Applicant : **CHUNG, Duane** [CA/CA]; Suite 3, 75 Maple Ave. S., Mississauga, Ontario L5H 2R7 (CA).

(72) Inventors: **PYNE, Michael**; 35 Elgar Ave., Hamilton, Ontario L9C 4E3 (CA). **YOUNG, Murray Moo**; Unit 14, 52 Blue Springs Drive, Waterloo, ontario N2J4M4 (CA). **CHOU, Chih-Hsiung**; 334 Craigleigth Drive, Waterloo, Ontario N2L 5B6 (CA).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to the identity of the inventor (Rule 4.17(i))*

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

[Continued on next page]

(54) **Title**: ELECTROTRANSFORMATION OF CLOSTRIDIUM PASTEURIANUM

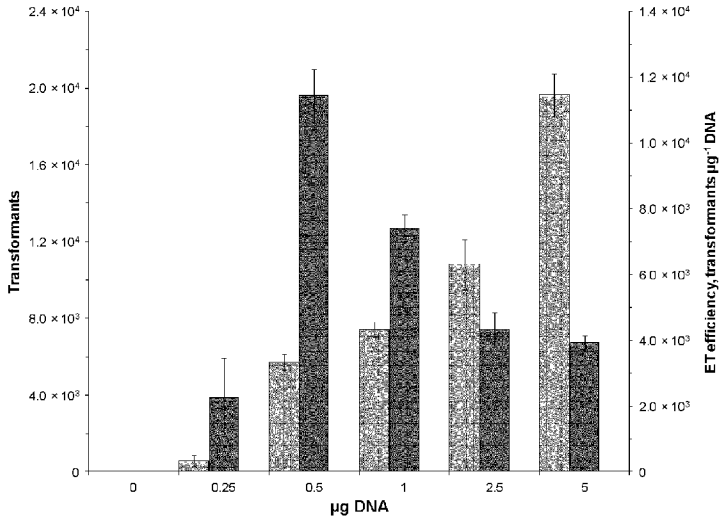


FIG. 6A

(57) **Abstract**: A method for high-efficiency genetic transformation of the anaerobic bacterium Clostridium pasteurianum is provided. Clostridium pasteurianum is industrially important due to its selective and high level production of the biofuel and bio-chemical n-butanol, and its ability to grow on a wide variety of inexpensive substrates. C. pasteurianum can use as a sole source of carbon and energy is glycerine, which is a by-product of biodiesel processing. The industrial exploitation of Clostridium pasteurianum has previously been impeded by the lack of genetic engineering tools for this bacterium. The method provided enables high-efficiency transformation of C. pasteurianum via a series of treatments and electroporation conditions which successfully negotiate the resistant cell wall of C. pasteurianum, and includes a means for protecting newly introduced DNA from degradation by a restriction enzyme within C. pasteurianum as well as selection markers and vector components.

WO 2014/107815 A3

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *the filing date of the international application is within two months from the date of expiration of the priority period (Rule 26bis.3)*

(88) Date of publication of the international search report:
4 September 2014